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# Emulsifying properties of succinylated arabinoxylan-protein gum produced from corn ethanol residuals

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ABSTRACT

This study investigated the possibilities of making valuable products from corn ethanol byproducts and providing the beverage industries more variety of high quality emulsifiers other than gum arabic. An arabinoxylan-protein gum (APG) was extracted from distillers' grains (DG), a low-value corn ethanol byproduct, and modified through acylation with succinic anhydride. The effects of pH and degree of substitution (DS) on the emulsifying properties of succinylated APG, referred to as SAPG, were investigated. Emulsion particle size and stability of APG and gum arabic were comparable at pH 3.5–6.5. Succinylation could enhance the emulsifying properties of APG. Compared to gum arabic, at pH < 5, SAPG emulsions had larger particle size but comparable stability, whereas at pH > 5, SAPG had much smaller particle size and better stability than gum arabic. The results suggested that SAPG, compared to gum arabic, could be a comparable emulsifier at low pH values and a better emulsifier at neutral pH values.


## 1. Introduction

Oil-in-water emulsions are common for many beverages with the oil droplets dispersed in the beverage's aqueous phase serving primarily as flavor oils (Chanamai & McClements, 2002; Given, 2009; Qian, Decker, Xiao, & McClements, 2011). Some non-flavor oils, such as omega-3 fatty acids, are also sometimes included to provide special nutrients (Given, 2009). Different from other food emulsions, beverage emulsions are very dilute (~0.2 wt% oil) and should have a great stability for a long period of time (Given, 2009; Yadav, Johnston, Hotchkiss, & Hicks, 2007). To disperse oil droplets in the aqueous phase, emulsifiers are needed to both facilitate the emulsion formation and stabilize the emulsion preventing the droplets from aggregating (Walstra, 1993).

The emulsifiers used in beverage emulsions are primarily amphiphilic polysaccharides due to their excellent emulsion stabilizing ability under changing environmental conditions (Chanamai & McClements, 2002; Garti, 1999; Qian et al., 2011). Gum arabic is the most commonly used in beverages due to its bio-based feature, food safety, high water solubility, low solution viscosity and great emulsion stability (Chanamai & McClements, 2002; Yadav et al., 2007). Gum arabic is a polysaccharides-protein complex with arabinogalactan as the main polysaccharides units; during emulsifying process, the hydrophobic protein portions anchor into the oil droplets, while the hydrophilic polysaccharides portions stick out into the aqueous phase, preventing the droplets from aggregation by steric or electrostatic effects (Chanamai & McClements, 2002; Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Jayme, Dunstan, & Gee, 1999; Randall, Phillips, & Williams, 1988; Walstra, 1993; Xiang, Anthony, Tobimatsu, & Runge, 2014a). Gum arabic is an effective emulsifier for beverage; however, the plants (*Acacia senegal*) producing gum arabic are primarily grown in the Sahelian region of Africa, and thus its supply is limited and price is sensitive to climate changes and is comparatively high to other food ingredients (Yadav et al., 2007).

As the demand for beverages and thus beverage emulsifiers increases, many studies have been conducted to find substitutes for gum arabic. Modified starch is common replacement beverage emulsifier for gum arabic (Chanamai & McClements, 2002).

*Abbreviations:* APG, arabinoxylan-protein gum; AXU, arabinoxylose unit; CI, creaming index; DG, distillers' grains; DS, degree of substitution; SA, succinic anhydride; SAPG, succinylated arabinoxylan-protein gum.

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Hydrophobic or non-polar groups are introduced onto starch through its abundant hydroxyls imparting it with both hydrophobic and hydrophilic portions similar to gum arabic; one of the examples is octenyl succinic acid (OSA) starch (Caldwell & Wurzburg, 1953; Chanamai & McClements, 2002; Viswanathan, 1999). Gum arabic has also been modified with octenyl succinic acid to increase the amount of its hydrophobic portion and reduce its usage level (Ward, 2002). Globular proteins, such as whey protein, are able to create oil droplets with size smaller than gum arabic during homogenization; however their stability is vulnerable to environmental condition changes (pH, temperature, etc.) (Chanamai & McClements, 2002; McClements, 1999; Qian et al., 2011). More recently, an arabinoxylan-protein gum (APG) extracted from corn ethanol byproducts was found with promising emulsifying properties (Xiang et al., 2014a; Yadav, Johnston, & Hicks, 2009; Yadav et al., 2007).

The U.S. produced approximately 56 billion liters of corn ethanol in 2014 yielding huge amount of fiber-protein rich byproducts (e.g. yielding ~40 million metric tons of distillers' grains) (RFA, 2015). Wet mill and dry grind are the two major processes in corn ethanol production industry in the US, between which dry grind is the most predominant process (Bothast & Schlicher, 2005; RFA, 2015). Many of the corn ethanol byproducts, such as corn fiber and distillers' grains (DG), from wet mill and dry grind processes respectively, are sold as animal feed at a very low price (Xiang & Runge, 2014). Therefore, effectively utilizing the proteins and polysaccharides in corn ethanol byproducts to make more valuable products could potentially increase corn ethanol profitability and sustainability (Xiang et al., 2014a; Xiang & Runge, 2014; Xiang, Watson, Tobimatsu, & Runge, 2014b).

Recently, an arabinoxylan-protein gum (APG) was extracted by alkali and/or $H_2O_2$ from corn fibers (Yadav et al., 2007, 2009) or distillers' grains (Xiang et al., 2014a). This gum is also an amphiphilic polysaccharide, similar to gum arabic (an arabinogalactan-protein complex). The structural differences between APG and gum arabic might be reflected by their chemical compositions as shown in Table 1. Other than the amphiphilic polysaccharide feature, the comparable molecular weight of APG with gum arabic (Table 1) also suggests the potential application of APG as beverage emulsifiers (Dickinson, Galazka, & Anderson, 1991). Arabinoxylan is a heterogeneous polymer and a major type of hemicelluloses found in corn (Gáspár, Kalman, & Reczey, 2007; Xiang et al., 2014b), with the backbone of β-(1-4)-D-xylopyranose being substituted primarily of α-L-arabinofuranose and slightly of D-galactopyranose and D-glucuronic acid (Ebringerová & Heinze, 2000; Xiang et al., 2014b). In corn, some of the hydroxyproline-rich glycoproteins are believed to link to the arabinoxylan through covalent bonds that were stable in alkali or $H_2O_2$ (Saulnier, Marot, Chanliaud, & Thibault, 1995; Xiang et al., 2014a; Yadav et al., 2007). Thus, some protein could be co-extracted with arabinoxylan as an arabinoxylan-protein complex and not be removed by the physical purification process (Xiang et al., 2014a).

**Table 1**
Composition (% based on oven-dried weight) and molecular weight of gum arabic, corn fiber APG and DG APG.

| Composition and molecular weight[a] | DG APG[b] | Corn fiber APG | Gum arabic |
|---|---|---|---|
| Arabinan (%) | 19 | ~30 | 20–50 |
| Galactan (%) | 5 | ~6 | 25–50 |
| Glucan (%) | 6 | ~1 | – |
| Xylan (%) | 28 | ~42 | – |
| Mannan (%) | 2 | – | – |
| Uronic acid (%) | 5 | ~4 | 10–20 |
| Protein (%) | 12 | 1–5 | 0.5–2 |
| $M_w$ (kDa)[c] | 450 | 300–450 | 300–900 |
| $M_n$ (kDa) | 62 | – | – |
| $M_w/M_n$ | 7.2 | 1.3–2.0 | – |
| References | Xiang et al., 2014a, b | Yadav et al., 2007, 2009 | Islam et al., 1997; Randall et al., 1988 |

[a] the compositions might be recalculated to represent the percent of oven-dried weight of the gums from the data provided by the references.
[b] the data for DG APG were from the composition and molecular weight for the specific batch of this study, and were consistent with the references provided in the table.
[c] $M_w$ is the weight average molecular weight, $M_n$ is the number average molecular weight, $M_w/M_n$ is the polydispersity index.

The corn fiber APG has been shown to have better emulsifying properties than regular or modified gum arabic (Yadav et al., 2007, 2009). The emulsifying properties of the DG APG were only comparable to gum arabic (Xiang et al., 2014a), and thus could be further improved through chemical modifications. Gum arabic or starch is typically modified with octenyl succinic acid to increase the amount of its hydrophobic portion (Ward, 2002). However, as shown in Table 1, the DG APG has protein content more than 10% (Xiang et al., 2014a), much higher than that of gum arabic (~1%) and corn fiber APG (1–5%) (Yadav et al., 2007, 2009). Additionally, gum arabic has 10–20% uronic acid (Islam et al., 1997; Randall et al., 1988) compared to ~5% in DG APG (Xiang et al., 2014a). Thus, it is hypothesized that chemically modifying the DG APG hydrophilic arabinoxylan portion with more steric or electrostatic effects might be an effective way to improve its emulsifying properties.

Acylation of polysaccharides with acid anhydrides is an effectively way to impart polysaccharides with carboxylic acid groups. High degree of substitution (DS) of acylation of polysaccharides with acid anhydrides is usually conducted in *N,N*-dimethyl formamide (DMF)/LiCl solutions with or without catalysts (Ebringerová, Hromádková, & Heinze, 2005; Lindblad & Albertsson, 2005; Sun, Sun, & Zhang, 2001). More recently, ionic liquids have also been used as an effective solvent (Hansen & Plackett, 2011; Peng, Ren, & Sun, 2010; Peng, Ren, Zhong, & Sun, 2011a; Ren, Peng, Feng, & Sun, 2013). Additionally, low DS acylations with acid anhydride were reported on starch and hemicelluloses by using alkaline solution as solvents (Betancur-Ancona, Garcia-Cervera, Canizares-Hernandez, & Chel-Guerrero, 2002; Jeon, Viswanathan, & Gross, 1999; Sun, Sun, & Bing, 2002). Avoiding the use of the organic solvents or ionic liquid would both economically and environmentally benefit the application of modified DG gum in food industries.

Therefore, to find suitable substitutes for gum arabic to relieve its limited supply, and to improve the value from the corn ethanol byproducts, we investigated acylating the arabinoxylan-protein gum (APG) from DG by succinic anhydride. The effects of DS and pH on the emulsifying properties of modified DG APG were quantified and compared to GA.

## 2. Materials and methods

### 2.1. Fractionation of APG

Distillers' grains (DG) samples were obtained from Didion Milling Inc. (Cambria, WI, USA). The arabinoxylan-protein gum (APG) was extracted and purified according to the procedure described previously (Xiang et al., 2014a). The DG was pre-extracted by acetone for 12 h in a Soxhlet extractor to remove fats and organic impurities and then was reacted in 3% (w/v) NaOH solution at 50 °C at 1:10 solid to liquid ratio (w/v) for 3 h. The separated alkali-soluble part was adjusted to pH 5.5, purified with bentonite clays to remove soluble proteins, and slowly poured into three volume of 95% ethanol with constant stirring. The precipitated solid was washed several times with 70% ethanol, freeze-dried, and ground to give a powder form of APG.

The extracted APG consisted of (based on the oven-dried

weight) 19% arabinan, 28% xylan, 5% galactan, 6% glucan, 2% mannan, 5% uronic acid, and 12% crude protein (Table 1). Number average molecular weight ($M_n$) of the APG was 62 kDa, weight average molecular weight ($M_w$) was 450 kDa, and the polydispersity index ($M_w/M_n$) was 7.2 (Table 1). The compositions and molecular weights of the APG were consistent with our previous studies, which provide a detailed characterization (Xiang et al., 2014a, b).

### 2.2. Succinylation of APG

APG was acylated by succinic anhydride (SA) based on the procedure modified from Sun et al. (2002). The unmodified APG was dissolved in 1% (w/v) NaOH solution to make a 5% (w/v) solution. Different amounts of SA (99.9%, Chem-impex Int'l Inc., IL, USA) leading to SA/arabinoxylose unit (AXU) mole ratios of 0.25, 0.33, 0.50 and 0.75 were added. The abbreviation AXU represents arabinoxylan mono sugar unit (anhydrous sugar), which has a molar mass of 132 g/mol assuming the arabinoxylan consists of only arabinose and xylose since the content of other minor units is very small (Xiang et al., 2014a, b). The mixture was adjusted to pH 8.5–9.0, and maintained at 30 °C with stirring for 1 h. The succinic anhydride modified DG arabinoxylan-protein gum (SAPG) was precipitated by pouring the mixture into three volumes of 95% ethanol and was washed twice with 70% ethanol solution and once with acetone in order to remove the unreacted SA.

### 2.3. Characterization of succinylated APG

The degree of substitution (DS) of a SAPG sample was defined as the mole of SA reacted per mole of AXU. The samples were coded as SAPGn, where the number n denotes the DS. The DS of SAPG was determined by measuring the amount of carboxylic acid group using a procedure modified from Stojanovic, Jeremic, Jovanovic, and Lechner (2005). A weighed SAPG sample of about 0.125 g was completely dissolved in 5 ml 0.2 M NaOH solution, to which 75 ml DI water was added. The excess NaOH was titrated with standard 0.05 M $H_2SO_4$ until a 3.75 pH was achieved. A blank sample was also titrated. The mole of COOH in the SAX sample was calculated as:

$$n_{COOH} = (V_b - V) \cdot 2 \cdot c_{H2SO4}$$

where $V_b$ is the volume (ml) of $H_2SO_4$ used for the titration of the blank; V is the volume (ml) of $H_2SO_4$ used for the titration of the sample; $c_{H2SO4}$ is the mole concentration (mol/ml) of $H_2SO_4$ used for the titration. Using the measured moles of COOH, the DS can be calculated as:

$$DS = \frac{132 \times n_{COOH}}{m_s - 100 \times n_{COOH}}$$

where 132 is the molar mass (g/mol) of an AXU; 100 is the increase in molar mass (g/mol) of an AXU for each SA substituted; $m_s$ is the oven dry mass (g) of the SAPG sample used for titration; $n_{COOH}$ is the mole of COOH.

$^{13}C$ NMR spectra of APG and SAPG samples were acquired on a Bruker Biospin (Billerica, MA, USA) AVANCE 700 MHz spectrometer fitted with a cryogenically-cooled 5-mm TXI gradient probe with inverse geometry (proton coils closest to the sample). Finely ball-milled samples (~50 mg) were swelled in $D_2O$ (1 ml). The spectra were recorded after 8000 normal scans and processed using Topspin 3.1 software (Bruker, Billerica, MA, USA).

Fourier transform infrared (FT-IR) spectra were obtained using a total of 10 scans using a Spectrum 100 FT-IR spectrometer (Perkin Elmer, Waltham, MA, USA) equipped with a universal ATR sampling accessory.

### 2.4. Emulsion preparation and emulsifying properties

APG, SAPG or a gum arabic sample (MP Biomedicals, Santa Ana, CA, USA) were dissolved (5 wt%) in the aqueous solution by using a sonicator for 30 min. The gum solution was then diluted 50 times into a 0.1 wt% gum solution and adjusted to pH = 3.5, 4.5, 5.5 or 6.5 by 5 wt% or 0.5 wt% citric acid. The gum solutions were homogenized with 0.5 wt% orange oil (Now Foods, Bloomingdale, IL, USA) at a high shear (20,000 rpm) in a Waring 700s Blender for 2 min and 2 times. The solutions were allowed to sit 5 min between the 2 blendings.

The surface-area-average diameter (droplet size, $d_{32}$) and zeta potential of the emulsions were measured right after preparations by a particle size analyzer (90Plus, Brookhaven, Long Island, NY, USA) according to the procedure described by Sun and Gunasekaran (2009). Cream stability test was conducted over a range of 8 days according to the procedure described by Keowmaneechai and McClements (2002). A creaming index (CI) from this procedure was recorded each day, which was calculated and as:

$$CI(\%) = \frac{H_S}{H_T} \times 100\%$$

where $H_T$ is the total emulsion height and $H_s$ is the serum layer height.

The rheological properties of the gum solutions were investigated. The dynamic (absolute) viscosity of the gum solutions was determined at 25 °C by using a rotational viscometer (Fungilab Adv Series, New York, USA) at rotational speeds of 1, 2.5, 5, 10, 20, 50, 100 rpm. Due to the viscometer's limited capacity, samples may not be measured at all selected speeds.

## 3. Results and discussions

### 3.1. Characterization of succinylated APG

Acylation of arabinoxylan with succinic anhydride (SA) would couple carboxylic acids to arabinoxylose units (AXU) through ester bonds (Fig. 1). To reach a high degree of substitution (DS) up to 1.0, the reaction should be conducted in DMF/LiCl with catalyst (Sun et al., 2001). However, this process might not be economical and might introduce toxicity for the final food grade products. Reaction of arabinoxylan with SA under mild alkaline condition (pH = 8.5–9.0) have been reported to be capable of controlling the DS below 0.2 (Sun et al., 2002), which was a more suitable modification method for this study.

The succinylated DG arabinoxylan-protein gum (SAPG) was first characterized by FTIR (Fig. 2). The peak at ~1735 $cm^{-1}$ of SAPG

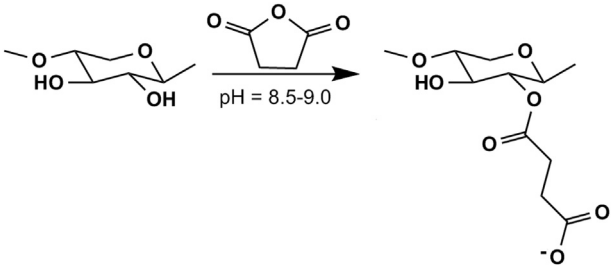


**Fig. 1.** Succinylation of arabinoxylan.

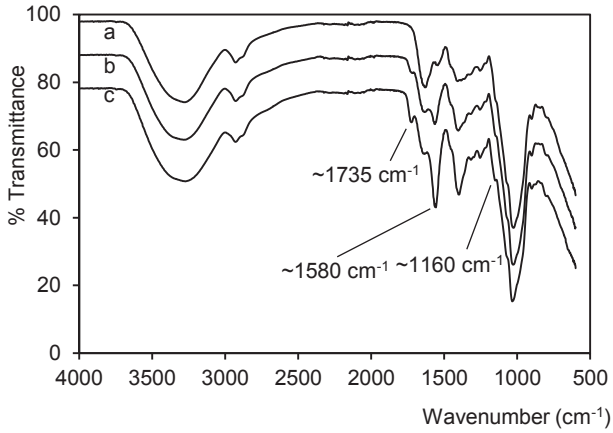


**Fig. 2.** FT-IR spectra of (a) APG; (b) SAPG with DS = 0.02; (c) SAPG with DS = 0.09.

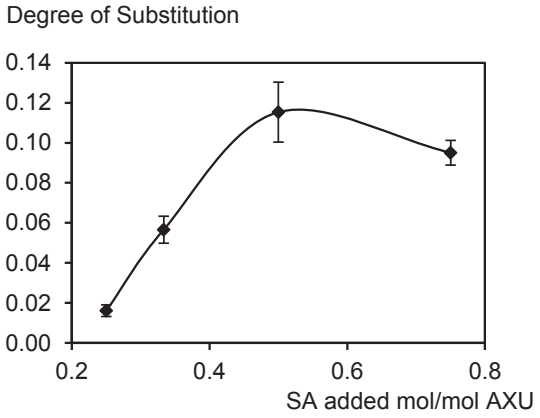


**Fig. 4.** Degree of substitution (DS) of SAPG vs. the amount of succinic anhydride (SA) added to the succinylation reaction.

corresponded to the C=O stretching of the ester bond or the carboxylic acid group, and the peak at ~1160 $cm^{-1}$ should be related the C–O stretching of the ester bond (Sun et al., 2001, 2002). The peak at ~1580 $cm^{-1}$ could include the N–H stretching of protein (Jagadeesh, Reddy, & Rajulu, 2001; Xiang et al., 2014b) and the asymmetric stretch of the carboxylates (Cabaniss, Leenheer, & McVey, 1998; Peng et al., 2011a). Additionally, the absence of signal between 1840 $cm^{-1}$ and 1760 $cm^{-1}$ indicated that the SAPG samples were free of detectable amount of unreacted SA. The $^{13}C$ NMR spectra (Fig. 3) of the SAPG were in consistent with the FTIR spectra. The peaks at 181.8, 178.0, and 176.3 ppm correspond to the carbons in the carbonyl of ester bonds and carboxylic acids confirming successful succinylation on the APG to SAPG.

The degree of substitution (DS) of the SAPG samples was varied using different SA/AXU mole ratio for the reaction (Fig. 4). The overall range of DS was between 0.02 and 0.12, which is consistent with the previous study (Sun et al., 2002). The DS increased as SA/AXU mole ratio increased, but when SA/AXU mole ratio went beyond 0.5, adding more SA did not have a positive response for increasing DS. Similar phenomenon was found when acylating starch with dodecenyl succinic anhydride in alkaline solution (Jeon et al., 1999). This may be due to insufficient mixing of SA and APG or insufficient reaction time for high extents of acylation under high SA concentration (Jeon et al., 1999; Sun et al., 2001). The low DS of the SAPG samples agreed with the low intensity of the carbonyl peaks from the NMR spectrum (Fig. 3). The increasing intensities of the FTIR spectra peaks at ~1160 $cm^{-1}$, ~1580 $cm^{-1}$ and ~1735 $cm^{-1}$ also agreed with the increasing DS of the analyzed SAPG samples (Fig. 2).

### 3.2. Particle size and zeta potential

Particle size (surface-area-average diameter $d_{32}$) is a key measure of the quality of an emulsion formed. During oil and water homogenization, three processes occur simultaneously: (1) large oil droplets are torn into smaller droplets; (2) the hydrophobic portion of the emulsifiers is absorbed onto the droplet surface; (3) oil droplets re-encounter each other, while the hydrophilic portion of the emulsifiers, which sticks out into the aqueous phase, prevents the droplets from aggregation through steric or electrostatic effects (Jayme et al., 1999; Walstra, 1993). Thus, oil droplet or particle size reflects both of how efficient the emulsifier is absorbed onto the oil droplet surface and prevents the droplet from aggregation. Zeta potential reflects the electrostatic repulsion between the adjacent droplet electrical double-layer and thus determines the electrostatic contribution to the stabilization of the emulsions (Jayme

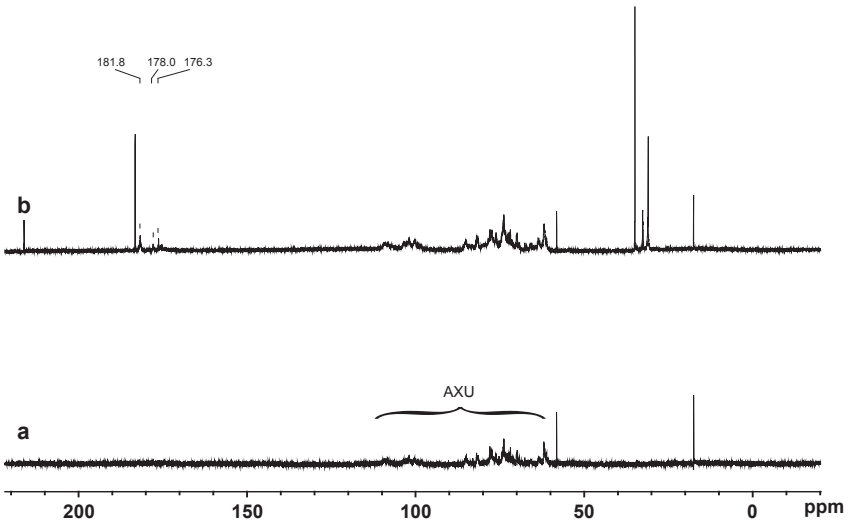


**Fig. 3.** $^{13}C$ NMR spectra of (a) APG and (b) SAPG with DS = 0.12.

et al., 1999).

The particle size ($d_{32}$) and zeta potential of oil-in-water emulsions stabilized by gum arabic, APG and SAPG at different pH (3.5–6.5) was measured, shown in Fig. 5. The particle size measurements of gum arabic emulsions were constant at ~1400 nm among different pH (Fig. 5a), which was consistent with previous studies (Qian et al., 2011). Emulsions stabilized by APG also demonstrated constant particle size over different pH, and the values were similar to those of gum arabic. These results suggested that APG had similar performance and likely would be useful in similar applications such as in assisting the formation of beverage oil-in-water emulsions, typically done under pH values ranging between 3.5 and 6.5.

The emulsions stabilized by SAPG with a low DS (0.02) also showed relatively stable particle size over different pH, but at a higher value of ~1800 nm, compared to gum arabic and APG (Fig. 5a). However, the particle size of emulsions stabilized by SAPG with a high DS (0.09) decreased significantly with increased pH, from ~1800 nm at pH = 3.5 and 4.5 to ~1200 nm at pH = 5.5 and ~800 nm at pH = 6.5. This phenomenon might be explained by the zeta potential changes of the emulsions (Fig. 5b). Compared to APG and SAPG0.02 emulsions at high pH (5.5 and 6.5), SAPG0.09 emulsion demonstrated significantly higher zeta potential indicating higher electrostatic repulsions between oil droplets. Gum arabic emulsion had high zeta potential at pH 6.5 too, but its particle size was constant at either high or low pH. The reason is that steric effects might be more prevalent than electrostatic repulsions in stabilizing the gum arabic emulsion (Jayme et al., 1999; Randall et al., 1988). However, for SAPG0.09 sample, as the zeta potential increased significantly and the particle size decreased significantly at pH = 5.5 and 6.5, it was clear that the electrostatic repulsion contributed significantly in stabilizing the SAPG0.09 emulsion. The electrostatic repulsion starting to take major effects at pH = 5.5 was likely due to that the succinic acid had pKa1 = 4.2 and pKa2 = 5.4, and thus at pH = 5.5, the carboxylic acid groups on the SAPG sample were greatly charged. It suggests that at pH > 5.5, the carboxylic acid group introduced by succinylation increased the charges of APG and the electrostatic repulsion, thus preventing oil droplets from aggregating.

Since at high DS, SAPG had better performance in stabilizing the emulsions, the effects of DS on the particle size of the SAPG emulsions were also plotted in Fig. 6. At pH = 3.5, as DS increased, the particle size slightly increased from ~1500 nm to ~1800 nm. While at pH = 5.5 and 6.5, the particle size increased slightly at DS = 0.02, and then decreased significantly at DS = 0.09. Continuing to increase the DS to 0.12 had limited effects on the particle size at pH = 5.5 and 6.5. It clearly indicated that at pH = 3.5 the carboxylic groups of SAPG had very limited charges due to their relatively high pKa and thus did not contribute to the electrostatic repulsion between oil droplets. When the DS increased beyond 0.09, it was postulated that adding more carboxylic groups was not effective in stabilizing the emulsions.

In summary, at a pH range between 3.5 and 6.5, APG had similar performance in forming emulsions compared to gum arabic. Succinylation imparted APG with better emulsion forming abilities at high DS and pH > 5, but reduced its emulsion forming abilities at low DS and pH < 5.

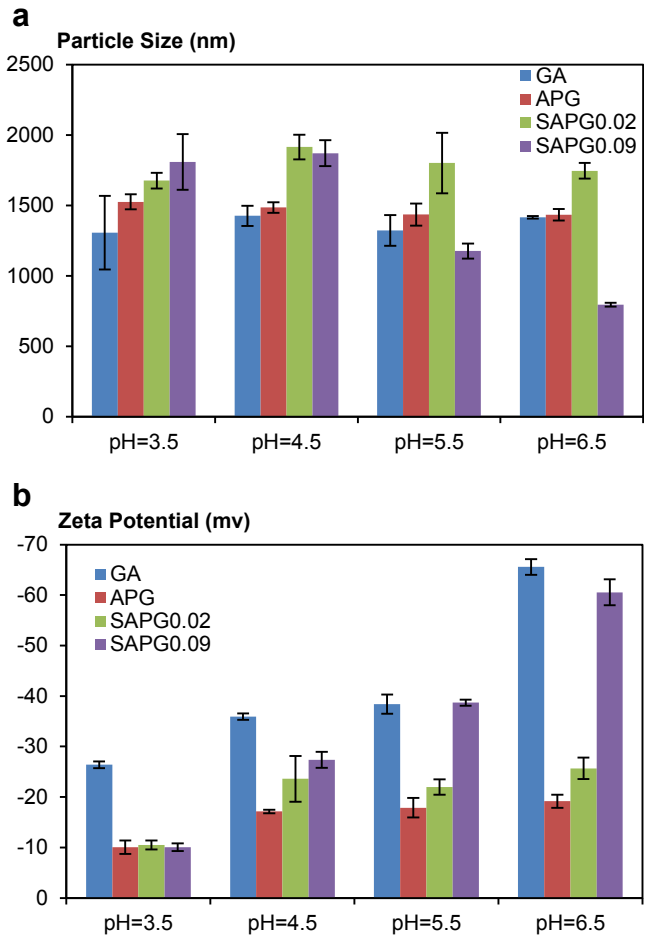


**Fig. 5.** (a) Particle size and (b) zeta potential of emulsions stabilized by gum arabic (GA), APG, SAPG0.02 and SAPG0.09 at pH 3.5 to 6.5 (e.g. SAPG0.02 means SAPG with DS = 0.02).

### 3.3. Emulsion stability

Emulsion stability is critical in many applications such as beverage emulsions, since many of these products are require to be stored for months or even years (Given, 2009; Yadav et al., 2007). Gum arabic is excellent in its emulsion stability but not in creating an initial small particle size. Some globular proteins are able to make emulsions with very small particle size, but very unstable (Chanamai & McClements, 2002; McClements, 1999; Qian et al., 2011).

The cream index (CI) reflects the emulsion's stability to creaming. The CI of the emulsions was recorded over a period of 7 days for the gum arabic, APG and SAPG stabilized oil-in-water emulsions at

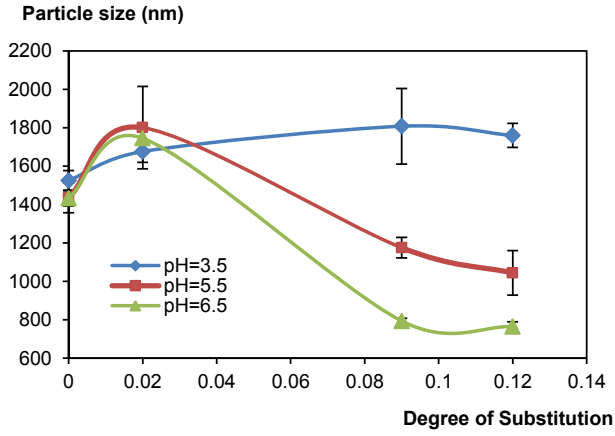


**Fig. 6.** The effects of degree of substitution (DS) on the particle size of emulsions stabilized by SAPG at pH 3.5, 5.5 and 6.5.

pH = 3.5 and 5.5 (Fig. 7). At pH = 3.5, graphs of CI of SAPG0.09 and SAPG0.12 emulsions overlapped with the gum arabic emulsion, while the graphs of APG and SAPG0.02 fell below gum arabic. It shows that at low pH, APG emulsions had better stability than those of gum arabic, and even though SAPG emulsions had larger initial particle size (Fig. 6), and their stability was still comparable to gum arabic. This phenomenon could possibly be explained by that, even though larger particle size should induce faster creaming (Keowmaneechai & McClements, 2002; McClements, 1999), the oil droplets with absorbed SAPG may have higher density due to the succinylation on APG and thus reduce the creaming rate (Sun and Gunasekaran, 2009). At pH = 5.5, APG and gum arabic emulsions demonstrated very similar stability, while all the SAPG sample, including SAPG0.02, which had the initial highest particle size among the all, had stability better than gum arabic and APG. This could also possibly be explained by the higher density of the oil droplets with absorbed SAPG compared to that with APG (Sun and Gunasekaran, 2009). On the other hand, at high pH, it was possible that the carboxylic groups on SAPG were greatly charged and the electrostatic repulsion between oil droplets was increased, hindering the flocculation and thus slowing down the creaming (McClements, 1999). Additionally, the higher molecular weight of APG than gum arabic (Table 1) may contribute to the APG or SAPG emulsion stabilities, since larger protein-containing macromolecules might be more responsible to the emulsion stability (Dickinson et al., 1991).

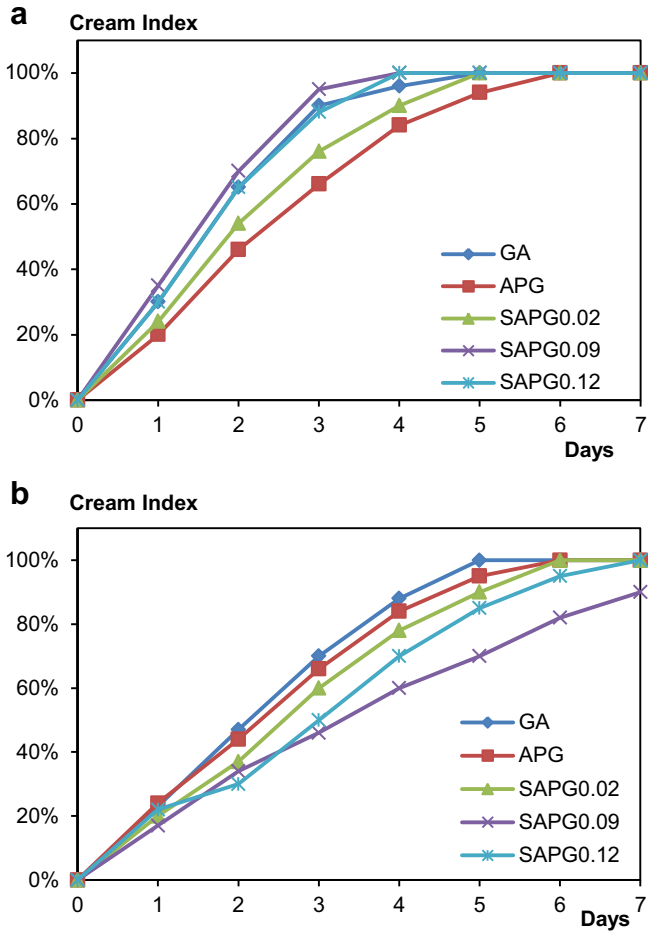


**Fig. 7.** Cream index over a period of 7 days for emulsions stabilized by gum arabic, APG and SAPG of different DS at (a) pH = 3.5 and (b) pH = 5.5 (e.g. SAPG0.02 means SAPG with DS = 0.02).

### 3.4. Viscosity of gum solutions

Viscosity is another characteristic that should be considered in evaluating beverage emulsifiers. Beverage emulsifiers might require low solution viscosity and gum arabic is well known for its low solution viscosities even at high concentration (Chanamai & McClements, 2002; Yadav et al., 2007).

In this study, the dynamic (absolute) viscosity of the gum solution was evaluated at 5 wt%, 2.5 wt% and 1.25 wt% concentrations, which was much higher than the concentration typically used in the emulsions (0.1 wt%). These higher concentrations were selected to accentuate the differences between the gums. Fig. 8 shows the dynamic (absolute) viscosity changes with shear rates by varying the rotational speed. At 5 wt% gum solution (Fig. 8a), APG demonstrated non-Newtonian fluid behavior, showing an apparent shear thinning phenomenon (shear rate) (Nishiwaki-Akine and Watanabe, 2014). SAPG0.12 sample behaved as a Newtonian fluid and had viscosity (~70 mPa s) lower than that of APG (~90 mPa s at high shear rate). At 2.5 wt% gum solution (Fig. 8b), both APG and SAPG sample behaved as a Newtonian fluid and showed a large drop in viscosity to ~16 and ~14 mPa s, respectively. At 1.25 wt% gum solution (Fig. 8c), the viscosity of APG and SAPG were ~6 mPa s. The non-Newtonian behavior of the APG solution was similar to the native wheat straw hemicelluloses (Peng, Ren, Zhong, Cao, & Sun, 2011b). The SAPG showing lower viscosity than the APG was in consistent with previous studies that succinylated cellulose and carboxymethyl hemicelluloses showed lower viscosity than their native forms (Li et al., 2009; Peng et al., 2011b), which was probably due the weakening of the intermolecular interaction between polysaccharide chains (Peng et al., 2011b). The significant changes of viscosity with solution concentration for APG and SAPG indicated strong hydrogen-bonding between their polymer chains or high entanglement at high concentrations (Peng et al., 2011b).

The dynamic viscosity of gum arabic solution was constant at ~5 mPa s among different studied concentrations, which was much lower than that of the APG and SAPG solutions at high concentration (Fig. 8), but approximately the same at concentrations < 1 wt%. These results suggested that the solution viscosity of SAPG was successfully reduced compared to APG making it more suitable to be used as a beverage emulsifier. Even though the solution viscosity of SAPG was still higher than gum arabic at high concentration, at low usage level (below 1 wt%) of emulsifiers, which is most likely the case for making beverages (Yadav et al., 2007), it might not be necessary to worry about the viscosity since APGS, APG and gum arabic are all showing similar values. At high concentrations, APG and SAPG could also be used as either a food thickener or an emulsifier. For some food products that are required to be thick and be a non-Newtonian fluid such as salad dressing (Tabilo-Munizaga & Barbosa-Canovas, 2005), APG might be a more promising food additive than SAPG.

## 4. Conclusions

Reacting the arabinoxylan-protein gum (APG) extracted from distillers' grains (DG) with succinic anhydride (SA) in mild alkaline solution, a succinylated APG (SAPG) was able to be produced without using organic solvents while allowing control of the degree of substitution (DS) to levels below 0.2. Between pH = 3.5 and 6.5, the unmodified APG was capable of creating dilute oil-in-water emulsions with particle size and stability comparable to gum arabic, with even better stability at pH = 3.5. SAPG emulsifying properties were more susceptible to pH. When pH < 5, SAPG created oil-in-water emulsions with larger particle size but comparable stability; however, at pH > 5, SAPG created emulsions with

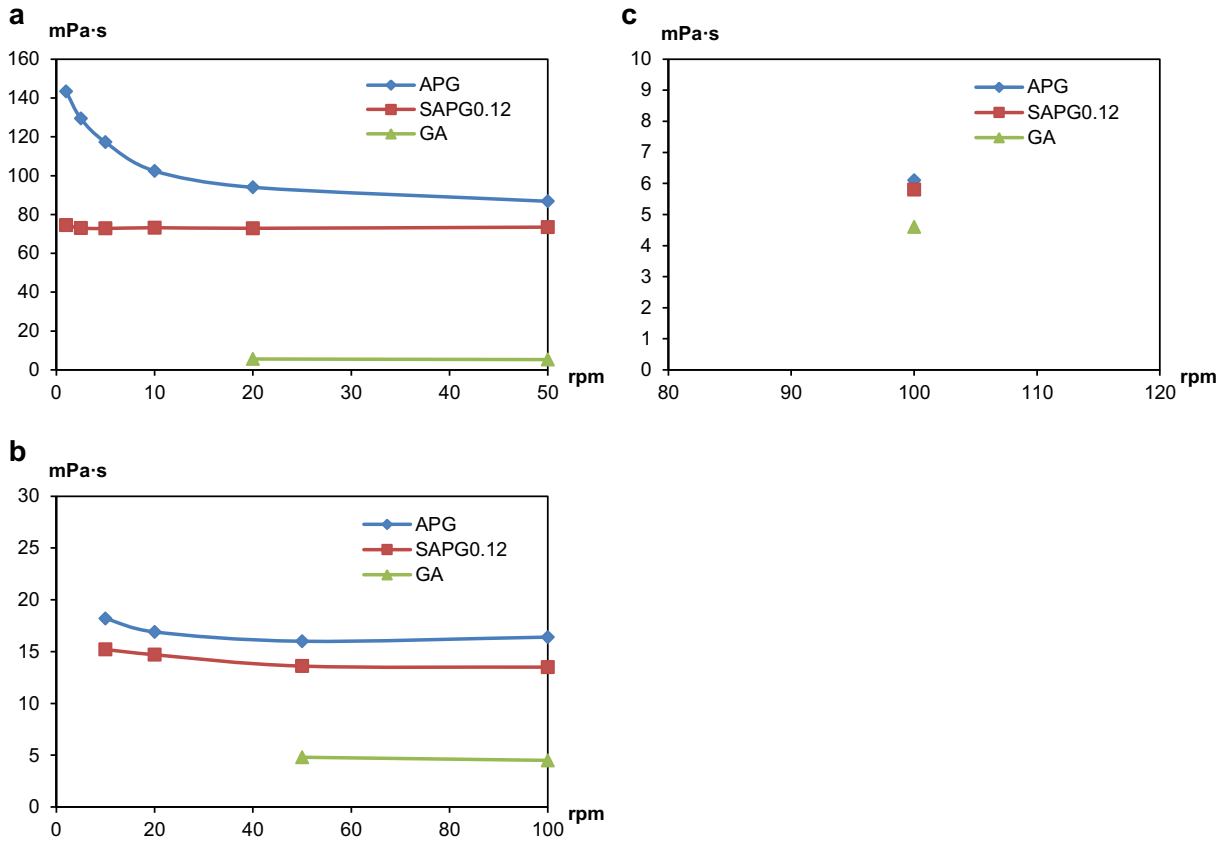


**Fig. 8.** Dynamic viscosity of (a) 5 wt%, (b) 2.5 wt% and (c) 1.25 wt% solutions of gum arabic, APG and SAPG with DS = 0.12 (Note: due to the viscometer's limited capacity, at 1.25 wt% gum solution, the viscosity could only be measured at 100 rpm).

much smaller particle size and better stability, compared to APG and gum arabic. Increasing the DS could further enhance the SAPG emulsifying properties, but beyond DS = 0.09, the DS effects were not significant. It may be postulated that the carboxylic acid groups introduced by the succinylation had pKa value of about 5, and thus at high pH, they were greatly charged providing highly improved electrostatic repulsions between oil droplets. This suggests that unmodified and succinylated APG can potentially substitute gum arabic as an emulsifier for producing beverage oil-in-water emulsions; while succinylated APG (SAPG), especially at high pH, was possibly a much better emulsifier than gum arabic.

The solution viscosity of APG was greatly reduced by succinylation, making SAPG a more suitable beverage emulsifier than APG from a viscosity standpoint. However, the non-Newtonian behavior of concentrated APG solution could possibly make it a food thickener especially for the food products required to be a non-Newtonian fluid.

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